Pediatrics and Neonatology (2015) 56, 382–385

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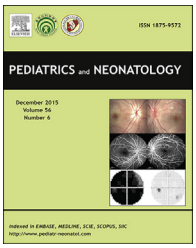

ORIGINAL ARTICLE

Angiogenic Factors in Cord Blood of Preterm Infants Predicts Subsequently Developing Bronchopulmonary Dysplasia

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Received Dec 8, 2014; received in revised form Jan 20, 2015; accepted Feb 9, 2015
Available online 20 April 2015

Key Words
bronchopulmonary dysplasia;
placenta growth factor;
soluble fms-like tyrosine kinase-1;
vascular endothelial growth factor

Background: Bronchopulmonary dysplasia (BPD) of prematurity is associated with impaired angiogenesis. Excess soluble fms-like tyrosine kinase-1 (sFlt-1) and lower levels of vascular endothelial growth factor (VEGF) impaired alveolarization in preterm rats. Overexpression of placenta growth factor (PlGF) in mice caused airspace enlargement, which is similar to BPD pathologically. Our study aimed to clarify whether cord blood levels of these angiogenic factors were associated with the development of BPD in preterm infants.
Methods: Preterm infants of gestational age (GA) <35 weeks who already had all the data of cord blood VEGF, PlGF, and sFlt-1 levels in our previous studies were enrolled. Cord blood levels of VEGF, PlGF, and sFlt-1 were collected. BPD was defined as the need for supplemental oxygen or mechanical ventilation support at the postmenstrual age of 36 weeks. We used the Mann-Whitney *U* test for comparison between infants with and without BPD, and multivariate analysis with logistic regression to assess the association of these molecules and the development of BPD.
Results: Infants with BPD had lower GA [(27 weeks (24–34) vs. 31 weeks (28–24)], lower birth body weight [882 g (620–1232) vs. 1538 g (886–2328)], a higher incidence of respiratory distress syndrome (RDS) (58% vs. 14%), and a higher level of PlGF [21.45 pg/dL (6.03 –474.01) vs. 7.43 pg/dL (0.09–23.75)] as compared with those infants without BPD. The levels of VEGF and sFlt-1 did not differ significantly between the two groups. Multivariate logistic regression revealed that lower birth body weight ($p = 0.022$) and higher level of PlGF ($p = 0.012$) were significantly correlated with the development of BPD independently. There was no significant association between the level of VEGF or sFlt-1 and the development of BPD.

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http://dx.doi.org/10.1016/j.pedneo.2015.02.001

Conclusion: Cord blood level of PlGF, rather than VEGF or sFlt-1, was significantly increased in the BPD group. Consistent with our previous report, cord blood level of PlGF may be considered as a biomarker to predict subsequently developing BPD in preterm infants.

1. Introduction

Bronchopulmonary dysplasia (BPD), a chronic lung disease followed by oxygen therapy and mechanical ventilator support, is one of the most common complications in preterm infants.[1] Indeed, it accounts for significant morbidities and mortalities for those who are born prematurely. With the introduction of antenatal steroid, exogenous surfactant, and greatly improved perinatal care, preterm infants who develop BPD are now more immature than formerly, and their clinical courses and pathological findings are also different.[2,3] Jobe[4] brought up the concept of "New BPD", which indicated that arrest of lung development instead of severe lung damage may presently be the major mechanism of BPD.

The causes of lung development arrest have been widely investigated. In recent years, ever more evidence and studies have suggested that appropriate angiogenic status is required for adequate pulmonary vascular development that could support normal alveolar lung growth.[5–7] Recently, Abman[8] proposed a vascular hypothesis that disruption of angiogenesis during lung development could impair normal lung growth including decreased alveolarization and decreased pulmonary arterial density, which were the typical characteristics of new BPD.

Vascular endothelial growth factor (VEGF) signaling is important for lung development. Inhibition of VEGF signaling led to abnormal pulmonary vascular growth and impaired alveolarization in several animal studies.[5,6,9–11] By contrast, excessive amniotic soluble fms-like tyrosine kinase-1 (sFlt-1), an endogenous VEGF antagonist contributing to the pathogenesis of preeclampsia, was documented to reduce alveolar number and arterial density in preterm rats.[9] In addition, placental growth factor (PlGF), a member of the VEGF family, mediates angiogenesis by modulating VEGF activity through competing to bind Flt-1. Besides its angiogenic effect, we found that overexpression of PlGF in transgenic mice resulted in increasing alveolar type II cell apoptosis that caused enlarged airspace and pulmonary emphysema, which is similar to BPD pathologically.[12] The aim of this study was to determine whether cord blood levels of these angiogenic or antiangiogenic factors were associated with the development of BPD.

2. Methods

2.1. Study participants

In our previous studies,[13–15] we studied the association between PlGF level of cord blood and the incidence of BPD,[13] VEGF level and the incidence of respiratory distress syndrome (RDS),[14] and sFlt-1 level and the platelet count in preterm infants.[15] Cord blood was collected using a heparinized syringe during delivery. After 15 minutes of centrifugation, the levels of VEGF, PlGF, and sFlt-1 were measured using a standardized sandwich enzyme-linked immunosorbent assay method as previously described.[13–15] In this study, preterm infants who were born less than gestational age (GA) of 35 weeks and who already had all the data of cord blood levels of VEGF, PlGF, and sFlt-1 from our previous studies, were enrolled. We excluded those infants with either prenatal maternal infection or neonatal infection within 3 days after birth. GA was defined by the means of last menstrual age or ultrasonography exams. Prenatal steroids were routinely administered during GA of 24 to 34 weeks when preterm labor was possible. We defined (RDS) as acute respiratory distress due to insufficiency of surfactant in the group of prematurity requiring higher concentration of oxygen and respiratory support based on radiographic characteristics. Under this condition, exogenous surfactant was administered via an endotracheal tube as quickly as possible when a fraction of >40% oxygen was required to maintain the blood oxygen level (SpO_2) up to 90%. As for BPD, the definition was the necessity for supplemental oxygen or any kind of ventilator support at postmenstrual age 36 weeks. We also collected all demographic information and perinatal history from a detailed chart review.

2.2. Data analysis

We used the Mann-Whitney *U* test for comparison between preterm infants with and without BPD, and multivariate analysis with logistic regression to assess the association of these molecules and the development of BPD.

3. Results

In total, 56 preterm infants were included in this study. Nineteen (34%) infants developed BPD. The BPD group had lower GA [27 weeks (24–34 weeks) vs. 31 weeks (28–24 weeks), $p < 0.001$], lower birth body weight [882 g (620–1232 g) vs. 1538 g (886–2328 g), $p < 0.001$], higher incidence of antenatal steroid usage (89% vs. 56%, $p = 0.012$), higher incidence of RDS (58% vs. 14%, $p = 0.001$), and longer period of intubation [27 days (0–109 days) vs 0 days (0–24 days), $p < 0.001$]. In addition, the BPD group had a higher level of PlGF as compared with those infants without BPD [21.45 pg/dL (6.03–474.01 pg/dL) vs. 7.43 pg/dL (0.09–23.75 pg/dL), $p < 0.001$]. However, the

levels of sFlt-1 and VEGF did not differ significantly between these two groups (Table 1).

According to our previous study, the cord blood level of PlGF was negatively correlated with GA,[13] so we performed multivariate analysis in order to clarify the importance of PlGF in the development of BPD. Multivariate analysis with logistic regression revealed that lower birth body weight ($p = 0.022$) and a higher level of PlGF ($p = 0.012$) were significantly correlated to the development of BPD independently (Table 2). As for VEGF and sFlt-1, there was no significant association with the development of BPD according to our analysis.

4. Discussion

In this study, we demonstrated that among these angiogenic and antiangiogenic factors, the level of PlGF rather than sFlt-1 or VEGF was significantly elevated in preterm infants with BPD. Consequently, the cord blood level of PlGF may be used as a predictor for subsequent development of BPD in preterm infants.

Exogenous surfactant replacement treats the functional immaturity for preterm lungs; however, it does not overcome the structural immaturity which remained a hallmark of lung development arrest.[16] Recent observations and studies emphasized the importance of angiogenesis and angiogenic factors during normal alveolar growth. Among these angiogenic and antiangiogenic factors that we investigated, the essential role of VEGF signaling was repeatedly emphasized. Several animal studies disclosed that inhibition of VEGF signaling indeed impaired alveolarization and disrupted normal lung growth.[5,6,9–11,17,18] Furthermore, alveolarization was enhanced when VEGF was additionally administered.[18–20] By contrast, sFlt-1 is an endogenous antagonist of VEGF signaling by capturing both VEGF and PlGF. From previous studies focusing on the relationship between sFlt-1 and preeclampsia, sFlt-1 was shown to have the antiangiogenic capability to cause a similar antiangiogenic state in the development of preeclampsia.[15,21–24] Recently, Tang et al[9] demonstrated that intra-amniotic injection of sFlt-1 resulted in reduced alveolar number and reduced pulmonary arterial density in fetal rat lungs. All these findings indicated that adequate angiogenesis is important for developing lungs. Besides the angiogenic effects, some reports documented that PlGF could also activate monocytes and result in increasing proinflammatory cytochemokines.[25,26] In addition, we previously demonstrated that PlGF overexpression transgenic mice had an enlarged airspace which was similar to pathologic findings of chronic obstructive lung disease in adult patients or BPD in preterm infants.[12] We also found that cord blood PlGF levels predicted poor pulmonary outcome in preterm infants.[13] By using PlGF knockout mice, we showed that elastase-induced pulmonary emphysema could be prevented by depleting PlGF *in vivo*.[27] These studies all suggested that PlGF may play an important role in chronic inflammatory lung diseases.

Therefore, low VEGF, high sFlt-1, and high PlGF levels tend to impair normal lung development. In our study, we found the cord blood levels of VEGF and sFlt-1 did not differ between preterm infants with or without BPD, although the importance of these two molecules was reported. The BPD group had significantly higher PlGF levels than the control group. Of course, this does not mean that high PlGF levels directly cause the subsequently developing BPD. Instead, the association suggests that cord blood level of PlGF may have the potential to be a useful predictor for the subsequently developing BPD in preterm infants.

The strength of this study is that it compares these three common angiogenic and antiangiogenic factors together to determine a biomarker to predict subsequently developing BPD. However, our study also has some limitations. First, this is not a case control study and the sample size is limited. We only enrolled the preterm infants who had all the data of cord blood PlGF, VEGF, and sFlt-1 levels.

Table 1 Characteristics and cord blood levels of angiogenic factors and antiangiogenic factors of preterm infants with and without bronchopulmonary dysplasia (BPD).

	BPD = 0 ($n = 37$)	BPD = 1 ($n = 19$)	p
GA	31 (28–34)	27 (24–34)	<0.001
BBW	1538 (886–2328)	882 (620–1232)	<0.001
Gender M:F	19:18	11:8	0.645
Prenatal steroid	20 (56%)	17 (89%)	0.012
RDS	5 (14%)	11(58%)	0.001
Surfactant	4 (11%)	6 (32%)	0.057
AS (1′)	6 (0–9)	6 (0–9)	0.513
AS (5′)	8 (0–10)	7 (5–9)	0.326
Intubation days	0 (0–24)	27 (0–109)	<0.001
sFlt-1	106.95 (35.8–1457.78)	146.04 (38.94–3600.46)	0.373
VEGF	18.821 (10.34–2390.6)	29.752 (11.81–311.7)	0.640
PlGF	7.43 (0.09–23.75)	21.45 (6.03–474.01)	<0.001

Data are presented as medians (ranges) and number (percentage).
AS = Apgar score; BBW = birth body weight; GA = gestational age; PlGF = placental growth factor; RDS = respiratory distress syndrome; sFlt-1 = soluble fms-like tyrosine kinase-1; VEGF = vascular endothelial growth factor.

Table 2 Factors associated with the development of bronchopulmonary dysplasia (BPD) in preterm infants.

	OR (95% CI)	p
GA	1.370 (0.991–1.894)	0.056
BBW	0.987 (0.976–0.998)	0.022
RDS	0.409 (0.28–6.045)	0.515
sFlt-1	0.998 (0.994–1.002)	0.254
VEGF	1.001 (0.997–1.004)	0.697
PlGF	1.515 (1.097–2.093)	0.012

BBW = birth body weight; CI = confidence interval; GA = gestational age; OR = odds ratio; PlGF = placental growth factor; RDS = respiratory distress syndrome; sFlt-1 = soluble fms-like tyrosine kinase-1; VEGF = vascular endothelial growth factor.

Therefore, there may be selection bias. The association between the PlGF level and BPD is consistent with our previous report.[13] Second, the definition of BPD used here is a traditional clinical definition but not a physiological definition, because this is a retrospective study. Therefore, a large prospective and randomized control study is warranted to confirm our finding.

5. Conclusion

Although perinatal care has improved significantly in recent decades, there was no effective and definite treatment for curing BPD or preventing BPD. In this study, we demonstrated that the cord blood level of PlGF rather than VEGF and sFlt-1 was significantly higher in preterm infants with BPD. This finding is consistent with our previous report which supported the idea that cord blood level of PlGF may be considered as a biomarker to predict subsequent developing BPD.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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